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JUVENILE HORMONE AND THE ADULT DEVELOPMENT OF *DROSOPHILA*

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It is clear that hormones control insect metamorphosis (see for example Wigglesworth, 1970; Wyatt, 1972; Doane, 1973), and that gene activity is involved (Ashburner, 1972a; Shearn, Rice, Garen and Gehring, 1971; Stewart, Murphy and Fristrom, 1972), but the precise interaction of the hormones with the genome is little understood. Williams and Kafatos (1971) have proposed a model involving juvenile hormone-controlled gene switching. Because of the hormone's involvement in genetic mechanisms, there is an increasing need for investigation of insect species which can be manipulated both genetically and cytogenetically. Genetic variations in *Drosophila* which alter endocrine functions have already been described. Juvenile hormone metabolism is altered in adults of a female sterile mutant (Postlethwait and Weiser, 1973), and several mutants affect ecdysone levels in mature larvae (Rayle, 1967; Karlson and Hanser, 1952).

Developmental aberrations have been described for *Drosophila* after treatment with juvenile hormone (JH) or certain JH analogs (Dearden, 1964; Bryant and Sang, 1968, 1969; Ashburner, 1970b; Madhavan, 1973). In these studies JH caused bristle modifications, failure of genitalia to rotate, and inhibition of eclosion. Interpretation of these effects on *Drosophila* has been complicated because there has as yet been no comparison of the abnormalities induced by exogenous application of JH analogues and the effects of juvenile endocrine organs implanted into ready-to-pupate hosts. This comparison is the first point studied in the present report: The developmental aberrations produced by the application of JH are compared to those provoked by the implantation of larval fragments containing living, active brain-ring gland complexes, the source of JH in the higher Diptera (Vogt, 1943, 1946).

If the genetics of *Drosophila* is to be exploited to help unravel the genetic basis of the hormonal control of insect development it would be best if a Dipteran juvenile hormone could be employed in such studies. But, if a Dipteran JH is to be characterized, a convenient and sensitive assay must be available. The second point approached by this communication is a JH assay for *Drosophila* which can detect as little as 2 picograms of Cecropia JH. The sensitive developmental period is defined, and several JH analogues are ranked with regard to their JH activity in *Drosophila* to provide information on the parts of the molecule important for biological activity.

Materials and Methods

Transplants

Drosophila virilis and *Drosophila melanogaster*, Oregon R wild strain, were cultured at 25° C using standard procedures. In order to test the effect of juve-

nile endocrine organs upon mature *Drosophila* larvae, first instar larvae 2–5 hours after hatching were cut into thirds in *Drosophila* Ringers (Chan and Gehring, 1971), and the anterior or posterior third was transplanted into a mature third instar host of the same species (see Ursprung, 1967, for transplantation techniques). Larval hosts were those which had ceased feeding and had crawled up onto the wall of the culture bottle to puparíate. In other experiments first instar brain-ring gland complexes, first instar mouth hooks, or late third instar imaginal discs were implanted into mature larval hosts. After metamorphosis the host was dissected and the position of the implant recorded. The cuticle of the host and the implant were then mounted in Gurr's water mounting medium between two cover glasses, and examined under 800 × magnification.

Staging for hormone treatments

For hormone applications developmental stage was reckoned with respect to puparíation—the time at which the larva becomes immobile, everts its anterior spiracles, and forms the puparium. The puparium remains white for only about a half hour (commonly called "white pre-pupa" stage); so this phase provides a convenient and accurate standard developmental stage. For experiments testing hormone sensitivity prior to puparíation, late third instar larvae were collected, anesthetized with ether, and treated with hormone or control solutions. Puparíated animals were collected hourly thereafter. For experiments testing hormone sensitivity after puparíation, white prepupae were collected and treated with hormone at appropriate times thereafter. A few pupae were etherized prior to acetone or hormone treatment. These animals gave results similar to those obtained without etherization; so in general, neither control nor experimental pupae were etherized.

Normal animals emerge from their puparia as adults four or five days after puparíation. Experimental animals were fixed in 70% ethanol seven days after puparíation, whether they had eclosed or not.

Application of solutions

Hormones were dissolved in Mallencrodt nanograde acetone. Controls were treated with acetone alone. Solutions were dispensed topically onto the dorsal posterior surface of the animals, except where noted in the text. Solutions were dispensed through a micro-pipette with a tip approximately 0.2 mm in diameter. A 100 μl Hamilton syringe was driven by a syringe pump for a calibrated period of time to deliver a convenient volume. Calibration showed that about 0.3 μl was delivered per dose.

Hormones

The following chemicals were tested according to the procedure described above: I., methyl 3,11-dimethyl-7-ethyl-*cis*-10,11-oxido-*trans,trans*-2,6-tridecadienoate (C18-JH); II., methyl 3,11-dimthyl-7-ethyl-*cis*-10,11-imino-*trans,trans*-2,6-tridecadienoate (C18-JH-aziridine); III., methyl 3,11-dimethyl-7-ethyl-*trans,trans, cis*-2,6,10-tridecadienoate (Methyl bishomofarnesoate); IV., 3,7,11-trimethyl-*cis, trans,trans*-2,6,10-dodecatrienoate (Farnesenic acid); V., 3,7,11-trimethyl-*cis,trans, trans*-2,6,10-dodecatrienol (farnesol); VI., methyl 3,7,11-trimethyl-*cis*-10,11-oxido-

trans,trans-2,6-tridecadienoate (C17-JH); VII., methyl 3,7,11-trimethyl-*cis*-10,11-oxido-*trans,trans*-2,6-tridecadienaldehyde (C17-JH aldehyde); VIII., methyl 3,11-dimethyl-7-ethyl-10,11-oxido-*trans,trans*-2,6-dodecadienoate (iso-C17-JH); IX., methyl 3,7,11-trimethyl-10,11-oxido-*trans,trans*-2,6-dodecadienoate (C16-JH); X., methyl 3,711-trimethyl-10,11-imino-*trans,trans*-2,6-dodecadienoate (C16-JH aziridine); XI., methyl 3,7,11-trimethyl-10,11-thio-*trans,trans*-2,6-dodecadienoate (C16-JH-episulfide); XII., methyl 7-chloromethyl-10,11-oxido-3 methyl-*trans,trans*-2,6-dodecadienoate (7 chloromethyl C16-JH); XIII., 3,7-dimethyl-6,7-oxido-*trans*-2-octenyl-3′,4′-methylene-dioxyphenyl ether (epoxygeranyl sesamole); XIV., 3,7-dimethyl-*trans*-2,6-octenyl-3′,4′-methylenedioxyphenyl ether (geranyl sesamole); XV., 3,7-dimethyl-6,7-oxido-*trans*-2-octenyl-4′-ethylphenyl ether (Stauffer R-20458); XVI., 3,7-dimethyl-6,7-oxido-*trans*-2-octenyl-4′-isopropylphenyl ether (Stauffer R-19828); XVII., methyl 3,7-dimethyl-6,7-oxido-*trans*-2-octenyl-4′-benzoate; XVIII., methyl 3,7-dimethyl-*trans*-2,6-octenyl-4′-benzoate; XIX., ethyl 7,11-dichloro-3,7,11-trimethyl-*trans*-2-dodecenoate (ethyl dichlorofarnesoate); XX., Law-Williams mixture (Law, Yuan and Williams, 1966); XXI., Sesamex; XXII., isopropyl 11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate, technical grade, (Zoecon ZR-515).

The author is grateful to Dr. Alfred Ajami for providing III, IV, VII-XI, XII-XX, (see Ajami, 1973, for procedures of synthesis and purification), to Professor E. J. Corey for providing I, II, VI and XII, and to Zoecon Corporation for XXII.

Data collection

The rotation of male genitalia was estimated in the stereo-microscope before dissection. The angle between the dorso-ventral axis of the fly and the axis of symmetry of the genitalia was estimated to the nearest 30°. Since the genitalia rotate clockwise during normal development, angles were estimated from 0–360° in a clockwise direction.

After measuring genitalia rotation, abdomens were removed from the animals, and internal structures dissected away. All cuticular structures including the head and thorax were then carefully mounted between two coverslips in Gurr's water mounting medium to check for hormonal effects at a magnification of approximately 800 ×. The surface architecture of the abdominal cuticles of a number of experimental and control *D. melanogaster* was studied in detail by scanning electron microscopy. Freshly prepared abdomens were examined after coating them *in vacuo* with carbon and gold-palladium.

The standard assay was 15 to 20 individuals per point in Figure 4. For the dose-response curves, each point represents two to four standard assays. The same individuals were used to study all four parameters of Figure 4, and where appropriate, in Figure 6. A total of 1071 C18JH-treated animals and 208 acetone treated controls provide the data for Figures 5 and 7. An additional set of 381 animals provide the data in Table III (the analogue assays).

Results

Effect of juvenile endocrine organs on the metamorphosis of Drosophila

The first series of experiments were designed to find the effect of juvenile endocrine organs upon the epidermis of a metamorphosing host. *D. virilis* and *D.*

TABLE 1

The effect of implanted parts of first instar larvae upon metamorphosing Drosophila virilis *hosts*

Implant	Age of implant	Number of recovered hosts	Number of hosts with aberrant cuticle above implant
Anterior third	First instar	84	17 (20%)
Posterior third	First instar	87	1 (1%)
Anterior third minus brain-ring gland complex	First instar	99	5 (5%)
Brain-ring gland complex	First instar	74	14 (19%)
Imaginal disc	Third instar	48	0 (0%)

melanogaster were used as donors and hosts. The data for both were similar, but were more extensive for *D. virilis*, and so these appear in Table I.

When abdomens of adult hosts were examined under the compound microscope, abnormalities were evident in 17 (20 per cent) of the 84 abdomens that had been implanted with the anterior third of first instar donors. Only 1 abnormality was encountered among 87 abdomens that had received the posterior third of first instar larvae. The abnormalities were localized in the cuticle immediately overlying the implant; they were encountered only when the implant was superficial, never when it was deeply imbedded in the abdomen. As illustrated in Figure 1, the

FIGURE 1. The effect of implanted juvenile endocrine organs on a metamorphosing host; A., Control adult abdominal cuticle from a host implanted with the posterior third of a first instar larva; B., Adult abdominal cuticle from a host implanted with the anterior third of a first instar larva; C., Normal pupal cuticle. Symbols used are AB., abnormal bristles; AC., aberrant cuticle; B., normal bristles; IC., imaginal cuticle; PC., pupal cuticle. Scale bar equals 100 μ.

abnormalities consisted of a localized zone closely resembling pupal cuticle and differing from adult cuticle in terms of the absence of pigment and hairs. So also, the bristles distinctive of adult cuticle were either absent or of aberrant size and shape.

Vogt (1946) apparently observed similar inhibition of adult differentiation after the implantation of *adult* corpora allata into maturing third instar hosts of *D. hydei*.

The ring gland of first instar larvae proved to be too small to be dissected and transplanted as such. Therefore, the brain-ring gland complex was removed and implanted into mature larval hosts (Table I). When the latter emerged as adults, 14 (19 per cent) of 74 individuals showed the typical integumentary defects. Here again, the local inhibition of metamorphosis was conditional upon the close proximity of implant and overlying cuticle. Control animals received the anterior third from which the brain-ring gland complex had been removed. Only 5 per cent of these animals showed the typical defect. This could be due to the retention of the ring gland by some of the anteriors since the ring gland grasps the pharynx rather tenaciously. As a final control imaginal discs from mature larvae were implanted into hosts of the same age, and none of these hosts showed any abnormalities.

These studies, coupled with Vogt's (1946) results, show that the ring gland of adult *Drosophila* and the brain-ring gland complex of first instar *Drosophila*

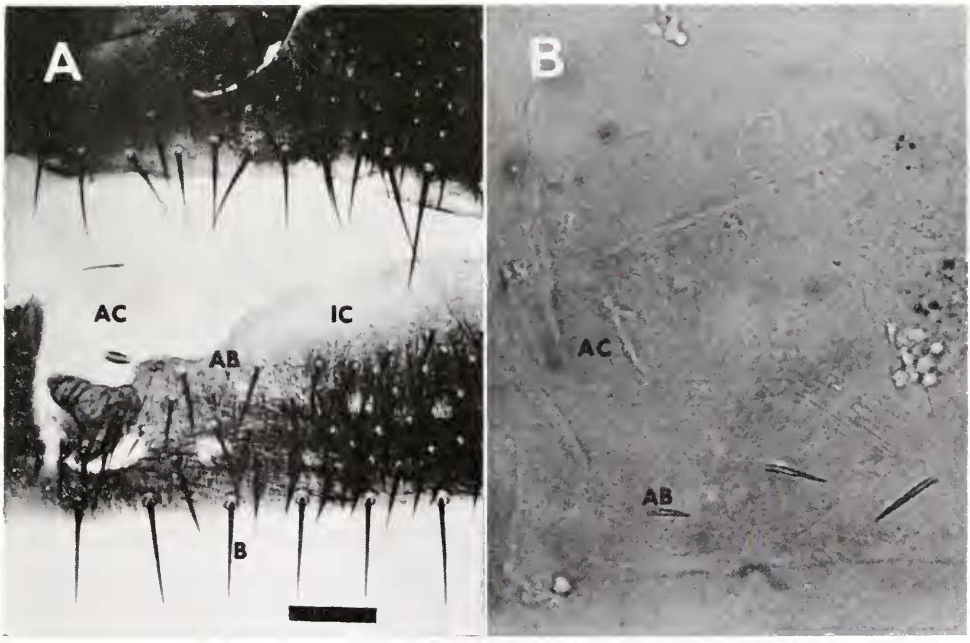

FIGURE 2. Effect of Cecropia juvenile hormone on the adult differentiation of the abdomen of *Drosophila;* A., 0.05 μg/animal; B., 5 μg/animal. Symbols used are: AB., abnormal bristles; AC., aberrant cuticle; B., normal bristle; IC., imaginal cuticle. Compare AC to Figure 1C. Scale bar equals 100 μ.

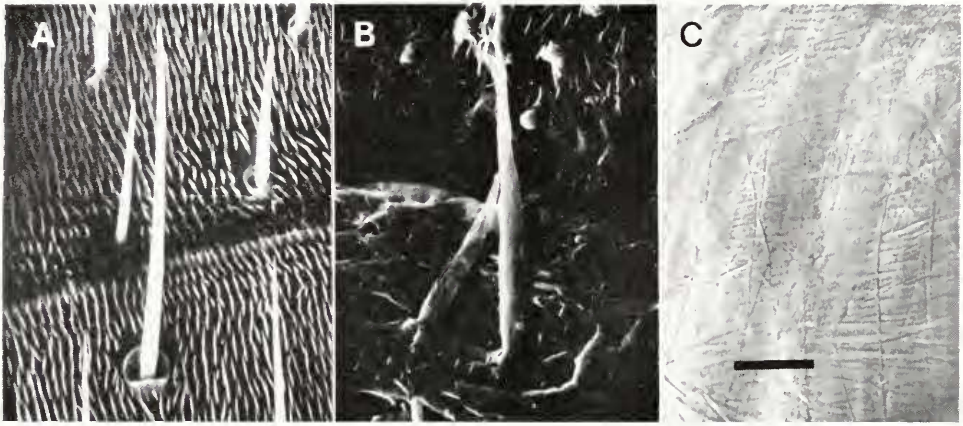

FIGURE 3. Scanning electron micrographs of: A., acetone treated control adult cuticle; B., juvenile hormone treated cuticle; C., pupal cuticle. Scale bar equals 20 μ.

cause local inhibition of abdominal metamorphosis. The aberrant cuticle is similar to pupal cuticle in that both lack pigment, bristles and hairs. In addition, surrounding the pupal-like regions are areas which have short, nicked, stubby or incompletely pigmented bristles.

The molting of the implants in these studies, published elsewhere (Postlethwait, 1973), confirm Bodenstein's (1944) results and show that larval tissues must develop competence to respond appropriately to the environment at metamorphosis.

Effect of exogenously supplied JH on the metamorphosis of Drosophila

Having ascertained the morphogenetic defects produced by implants containing juvenile endocrine organs, we centered further attention on the effects of topically applied, synthetic JH. Individuals receiving in excess of 0.05 μg C18JH formed defective pharate adults which failed to emerge. As illustrated in Figures 2 and 3, the abdominal tergites of individuals receiving low doses of JH showed patches of cuticle identical to those produced by the implantation of brain-ring gland complexes. In the case of individuals treated with the highest doses, the entire "adult" abdomen was covered by an aberrant cuticle showing an almost complete suppression of pigmentation and of bristles and hairs (Fig. 2B). The cuticle in the affected areas was impressively different from that of the controls (Fig. 1) and in many cases was indistinguishable from pupal cuticle over large areas. Although the aberrant cuticle could not be distinguished from pupal cuticle in whole mounts, it must be noted that the normal pupal cuticle lacks any projections or irregularities which in unstained whole mounts permit its positive and unambiguous identification.

But we can conclude that exogenously supplied synthetic JH causes a general syndrome identical to that produced locally by an implanted active young larval brain-ring gland complex, and in both cases the aberrant cuticle is indistinguishable from pupal cuticle.

Developmental stages during metamorphosis which are most sensitive to exogenously supplied JH

Effects on adult eclosion. Figure 4A records the percentage of flies which failed to eclose when specific doses of C18JH were administered at discrete ages ranging from the mid-third instar until one day after the initiation of pupariation. All except the lowest dose had major effects in blocking eclosion when adminis-

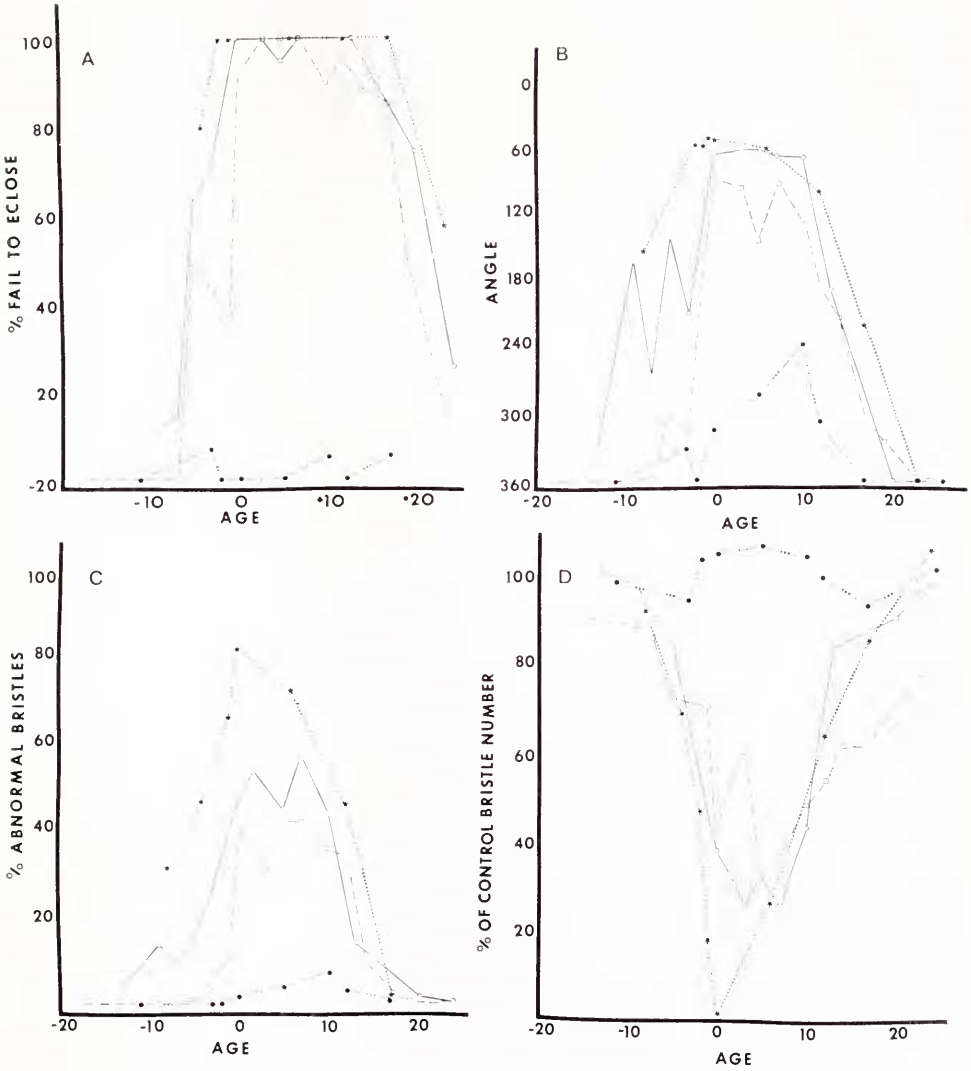

FIGURE 4. Four parameters of JH activity and their relationship to age at time of treatment; A., per cent failing to eclose *vs.* age; B., failure of male genitalia to rotate; C., per cent abnormal bristles; D., per cent of bristles missing on the male 5th tergite and female 6th tergite; Stars, 5.0 μg; open circles, 0.5 μg; squares, 0.05 μg; filled circles, 0.005 μg.

tered during a certain critical period. The latter begins about 5 hours prior to the onset of pupariation and persists for approximately 25 hours thereafter. Maximal sensitivity includes the first 12 hours after pupariation.

Effects on the rotation of the male genitalia. During a terminal phase of adult differentiation of Dipterous insects the male genitalia undergo a permanent rotation with respect to the long axis of the body (Scudder, 1971). In the lower Diptera the rotation is through 180°; in the higher Diptera such as *Drosophila,* it is through 360° (Gleichauf, 1936). Genitalia rotation is partially or completely blocked in mosquitoes (Spielman and Williams, 1966) and *Sarcophaga* (Bhaskaran, 1972) derived from pupae exposed to JH analogues; the same is true for *Drosophila* according to Bryant and Sang (1968) and Madhavan (1973).

These findings are confirmed in the present study. Thus, in Figure 4B, is recorded as a function of dosage and time of application the per cent by which the male genitalia failed to undergo the normal 360° rotation. Here again, the period of maximal sensitivity is the first 12 hours after the initiation of pupariation.

Effects on bristle number and morphology. In order to quantify the inhibition of metamorphosis, a detailed study on the 5th abdominal tergite of males and the 6th of females was undertaken. These segments were chosen because they have both bristles and hairs (trichomes) and because the posterior tergites are affected to a greater degree than the anterior tergites. The male 5th tergite is characterized by 54 ± 4 bristles; the female 6th tergite by 62 ± 3 bristles in normal flies or in controls treated only with acetone. Counts were made of the total number of bristles which had differentiated on these tergites, as well as the number which were abnormal.

Figure 4D records the percentage of bristles (with reference to control values) as a function of dose and time of application. In Figure 4C is plotted the percentage of abnormal bristles among those which had differentiated. Here again, we see that the effects are dose-dependent and that the sensitive period is from shortly before to about 15 hours after the initiation of pupariation.

Effect of dose and site of application on integumentary defects

Topical application of the JH doses employed was not able to suppress the normal adult differentiation of the integument of the head or thorax. Only an occasional individual which had received the highest dose (5 μg) at the most sensitive stage showed a few abnormal bristles on the head, but no other trace of abnormal characters on either the head or thorax. By contrast, the adult differentiation of the abdomen was subject to inhibition by JH when the latter was applied during the sensitive period. The typical result was the formation of more or less extensive zones of unpigmented, hairless cuticle in which the bristles were either missing or of aberrant size and shape. Featureless zones within this cuticle were indistinguishable from pupal cuticle as shown above. These characteristics are illustrated in Figures 5B–5E in the case of four individuals that received graded doses of JH in the "white puparium" stage. For comparison, a normal adult integument is depicted in Figure 5A and a normal pupal integument in 5F.

In the experiments summarized in Figure 6, graded doses of C18-JH were applied to individuals at a single highly sensitive stage—namely, at the outset of

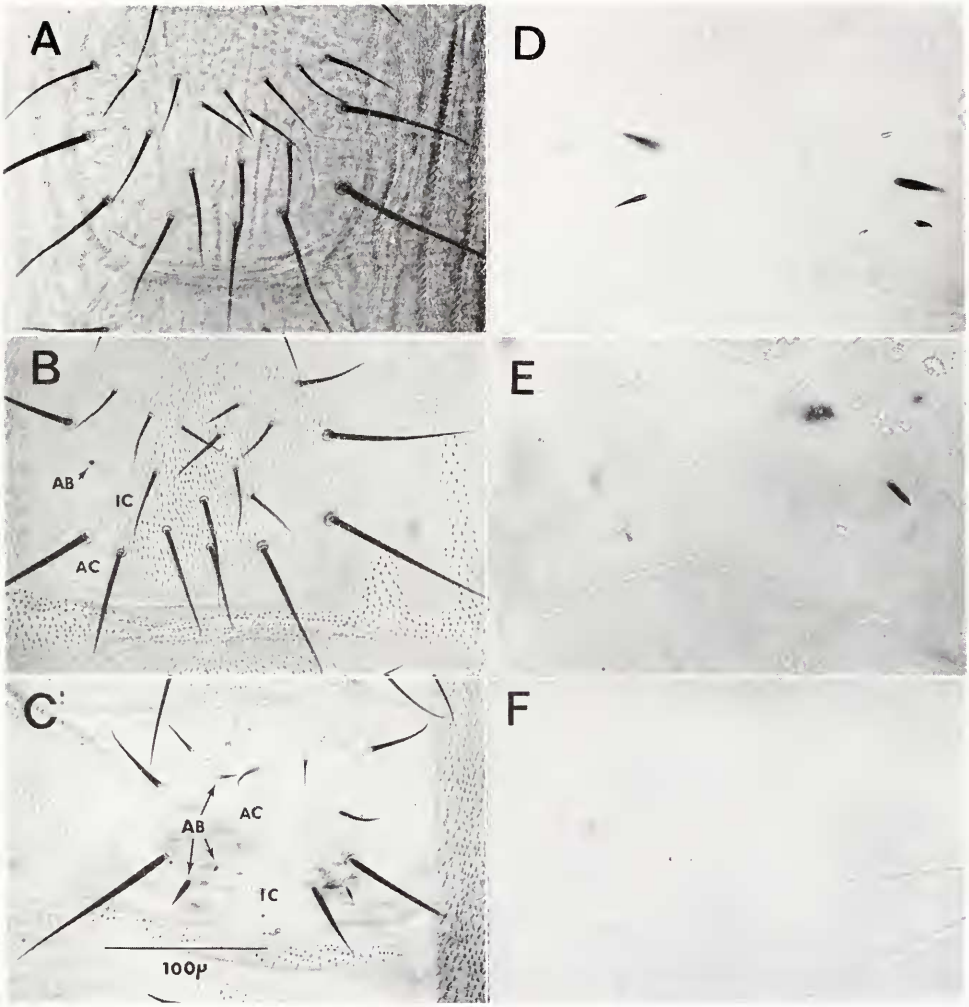

FIGURE 5. Affect of increasing doses of C18JH on the sternites of *Drosophila;* A., acetone-treated control; B., 0.002 μg; C., 0.005 μg; D., 0.05 μg; E., 0.5 μg; F., Pupal cuticle; AB, abnormal bristles; AC, aberrant cuticle; IC, imaginal cuticle.

pupariation (white puparia). The several parameters of juvenilization are in each case plotted as a function of the logarithm of dose. Table II records the threshold doses, the saturating doses, and the doses that give 50% effects.

When low doses of hormone were administered during the critical period, the posterior abdominal segments were more sensitive than the anterior segments and the abdominal sternites were routinely more effected than the tergites. Meanwhile, as mentioned above, the head and thorax remained insensitive at all stages.

The insensitivity of the head and thorax was examined in two series of experiments. In the first of these the hormone was applied to the anterior ends of white prepupae rather than to the posterior ends. This shift in the site of application

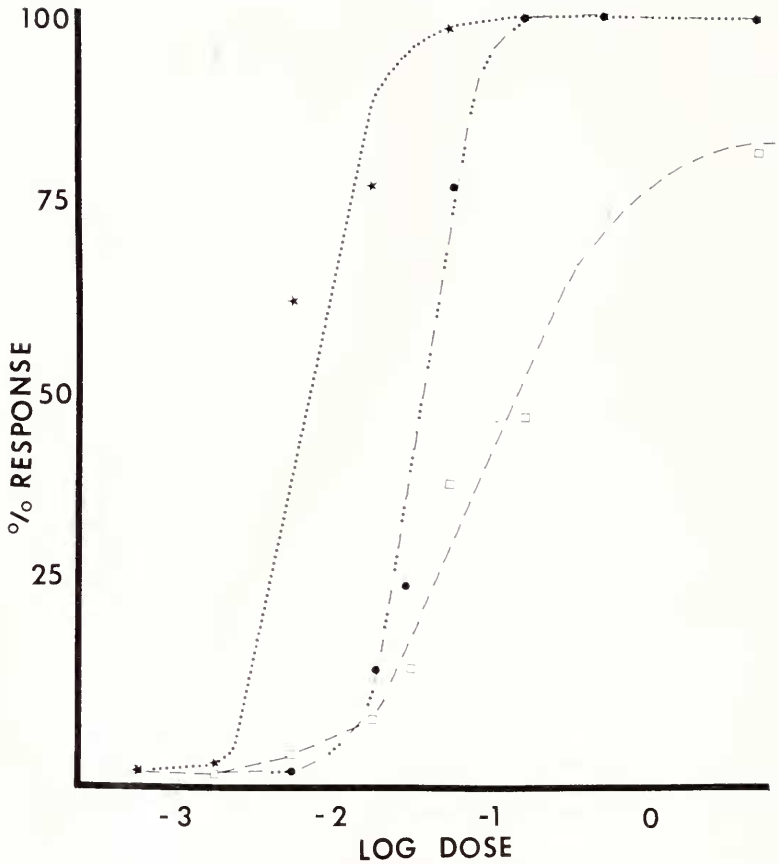

FIGURE 6. Dose response curves: stars, abnormal sternite bristles; filled circles, failure to eclose; squares, abnormal tergite bristles.

had no effect on the differentiation of the adult head and thorax. Moreover, the effect on the abdomens was indistinguishable in the two cases except that the rotation of the male genitalia was somewhat more inhibited in the individuals that received the hormone at the hind end.

TABLE II

Quantitative aspects of the action of C18 JH on white puparia

Effect	(Critical doses (μg/individual)*		
	Threshold	50% Effect	100% Effect
Abnormal bristles on sternites	0.002	0.006	0.05
Failure to eclose	0.016	0.03	0.2
Inhibition of rotation of genitalia	0.005	0.03	0.2
Abnormal bristles on tergites	0.005	0.6	0.75

* Each dose was topically applied in 0.3 μl acetone.

TABLE III

Relative abilities of JH analogues to block metamorphosis

Approximate rank	Analogue Number	Designation	Concentration μg/g	# of animals tested	% fail to eclose	% with morphogenetic effect on sternites	Degrees male genitalia rotated	% control bristle #	% aberrant bristles per tergite
1	XIII	epoxy geranyl sesamole	34	28	100	100	15 ± 12	36 ± 18	95 ± 10
2	VIII	iso-C17-JH	34	15	87	100	54 ± 40	70 ± 17	55 ± 20
3	XXII	ZR-515	34	22	100	100	100 ± 33	66 ± 21	65 ± 34
4	IX	C16-JH	34	15	60	100	67 ± 36	90 ± 12	39 ± 20
5	I	C18-JH	34	47	76	100	87 ± 39	82 ± 15	36 ± 16
	1	C18-JH	17	15	27	100	240 ± 100	97 ± 13	19 ± 13
6	I + XXI	C18-JH + sesamex	17	15	33	100	200 ± 70	103 ± 10	13 ± 7
7	VI	C17-JH	34	15	47	100	154 ± 52	91 ± 15	18 ± 15
8	XV	R-20458	34	15	60	100	154 ± 79	102 ± 10	19 ± 9
9	XVI	R-19828	34	15	93	100	300 ± 42	104 ± 14	8 ± 3
10	XIV	geranyl sesamole	34	15	67	100	283 ± 67	104 ± 10	5 ± 4
11	XII	ClMe-C16-JH	34	13	0	100	265 ± 86	108 ± 11	4 ± 4
12	XI	C16-JH-S	34	15	0	100	308 ± 66	110 ± 11	4 ± 5
13	III	MBHF	34	15	0	100	342 ± 40	103 ± 14	1 ± 2
14	XX	Williams-Law	34	15	0	78	360 ± 0	107 ± 11	0.4 ± 1
15	XIX	EDCF	34	15	0	29	360 ± 0	102 ± 11	0.2 ± 0.8
16	IV	Farnesenate	34	14	0	43	356 ± 11	102 ± 10	0.1 ± 0.5
17	V	Farnesol	34	14	0	50	360 ± 0	102 ± 9	0 ± 0
18	II	C18-JH-N	34	14	7	14	360 ± 0	108 ± 10	0.1 ± 0.1
19	X	C16-JH-N	34	14	0	0	360 ± 0	110 ± 8	0 ± 0
20	VII	C17-JH-aldehyde	34	15	0	0	360 ± 0	98 ± 7	0 ± 0
21	XVII	Epoxy geranyl methyl benzoate	34	14	0	0	360 ± 0	102 ± 9	0 ± 0
22	XVIII	geranyl methyl benzoate	34	10	0	0	360 ± 0	98 ± 12	0 ± 0
23	XXI	Sesamex	34	15	0	0	360 ± 0	100 ± 12	0 ± 0
24		Acetone		26	0	0	360 ± 0	100 ± 9	0 ± 0

In the second series of experiments the operculum was removed from puparia and the hormone was applied directly to the pupal head. (This operation cannot be carried out before the 12th hour since, prior to that time, the epidermis is firmly attached to the operculum.) In each of the treated individuals the head and thorax underwent essentially normal adult development. A few bristles were bent abnormally, but this occurred also on animals whose operculum was removed and who were then treated with acetone. The effects on the abdomen could not be distinguished from that seen in controls in which the hormone was applied to intact puparia of the same age.

The relative abilities of different JH analogues to block metamorphosis

As a check on the general types of molecules which have JH activity in *Drosophila*, I compared the morphogenetic effects of 22 different JH analogues (Table III). The conclusions drawn below are subject to the fact that the isomer composition of most of the compounds tested is unknown. The study shows that for *Drosophila* the synthetic JH analogues epoxy geranyl sesamole and ZR-515

are both more active than any of the hormones isolated from Lepidoptera (compounds I, VI and IX, Roller, Dahm, Sweely and Trost, 1967; Meyer, Schneiderman, Hanzmann and Ko, 1968; Dahm and Roller, 1970; Judy, Schooley, Dunhan, Hall, Bergot and Siddall, 1973). A comparison of JH analogue activities shows that for *Drosophila* a methyl group at position 11 is better than ethyl since iso-C17-JH (VIII) is more active than C18-JH (I), and C16-JH(IX) is more active than C17-JH (VI). An epoxy ring is better than a double bond between carbons 10 and 11 since C18-JH (I) is more active than methyl bishomofarnesoate (III) and epoxy geranyl sesamole (XIII) is more active than geranyl sesamole (XIV). An epoxy ring is better than an imino ring or a thio ring between carbons 10 and 11 since C16-JH (IX) is more active than C16-JH-aziridine (X) or C-16-JH-episulfide (XI) and C18-JH (I) is more active than C18-JH-aziridine (II). An ethyl group at position 7 is better than a methyl since iso-C17-JH (VIII) is more active than C16-JH (IX) and C18-JH (I) is more active than C17-JH (VI). A methyl group at position 7 is better than a chloromethyl since C16-JH (IX) is more active than chloromethyl-C16-JH (XII). Sesamole is more active than other modified benzenes in an ether link to geranol since epoxy geranyl sesamole (XIII) is more active than R-20458 (XV), R-19828 (XVI) or epoxy geranyl methyl benzoate (XVII).

Discussion

These experiments show that a characteristic syndrome of abdominal cuticular abnormalities can be induced in *Drosophila* by either implanting a first instar brain-ring gland complex into a mature larva, or by topically treating a freshly pupariated *Drosophila* with Cecropia juvenile hormone. The characteristics of the aberrant cuticle involve a failure of the cuticle to pigment, or to form cell hairs. Surrounding these areas are bristles which are short, nicked, or incompletely metamorphosed. The bristle irregularities confirm the results of earlier workers (Dearden, 1964; Bryant and Sang, 1968, 1969; Ashburner, 1970b; Madhavan, 1973).

Since the entire brain-ring gland complex (including several endocrine organs) was transplanted, the precise inducer of the aberrant cuticle is in question. But the evidence is consistent with the interpretation that the corpus allatum part of the ring gland inhibits metamorphosis. First, either ring glands or brain-ring gland complexes of *Calliphora* when transplanted into fifth instar hosts of *Rhodnius* induce the retention of larval cuticle in the adult (Wigglesworth, 1954). Secondly, adult corpora allata implanted into mature larval hosts inhibit normal adult development of the abdomen in *Drosophila hydei* (Vogt, 1946) or *Calliphora* (Possompes, 1953), causing the same syndrome as appeared in our experiments. Thirdly, first instar ring glands of *D. hydei* implanted into adult females which had been deprived of their ring glands induced vitellogenesis, whereas brains alone failed to induce egg maturation (Vogt, 1943). It therefore seems that the ring gland of flies produces a juvenile hormone, and it may be that transplanted young ring glands caused the aberrant cuticle in our transplants.

The experimentally induced aberrant cuticle was indistinguishable from pupal cuticle, but that does not prove that it *is* pupal cuticle. Since pupal cuticle is

devoid of unique and specific cuticular markers, its positive identification using purely morphological criteria is frustrated.

Five higher flies other than *Drosophila* have been tested for their response to juvenile hormone analogues: *Sarcophaga bullata* (Srivastava and Gilbert, 1968, 1969; Bhaskaran, 1972), *Sarcophaga argyrostoma* (Fraenkel and Hisao, 1970), *Calliphora erythrocephala* (Pihan and Bautz, 1970), *Stomoxys calcitrans* (Wright, 1970; Wright and Rushing, 1973; Wright, Crookshank and Rushing, 1973), and *Musca domestica* (Herzog and Monroe, 1972; Cerf and Georghiou, 1972). These studies showed that JH analogues inhibited eclosion and metamorphosis and suppressed the differentiation of hairs, bristles and pigment characteristic of the adult fly. In three of these investigations histological sections were prepared. Srivastava and Gilbert (1968, 1969) interpreted their sections as showing two pupal cuticles, the second apparently being induced by JH. Wright (1970) and Bhaskaran (1972) found no second pupal cuticle nor a pupal-adult intermediate cuticle. Bhaskaran (1972) suggested that the "second pupal cuticle" described by Srivastava and Gilbert (1968, 1969) may be undifferentiated adult cuticle or the undigested inner layers of the first pupal cuticle. The latter explanation could not hold for the pupal-similar cuticle reported here for *Drosophila* since the normal and aberrant cuticle are contiguous in treated abdomens. The aberrant cuticle in *Drosophila* may be merely undifferentiated adult cuticle which, by chance, is indistinguishable from pupal cuticle. The available data do not allow us to distinguish between the possibilities that in *Drosophila* juvenile endocrine glands and synthetic Cecropia JH induce a second pupal cuticle in the abdomen, or rather an aberrant adult cuticle that cannot be distinguished from pupal cuticle in whole mounts. Biochemical or immunological identification of pupal-specific cuticular protein could resolve the dilemma.

The procedure developed here provides a simple quantitative method for the assay on *Drosophila* of substances suspected to possess JH activity. This test is a sensitive measure of JH activity—as little a 0.0015 μg C18-JH can be detected. The dose-response curves show that for the posterior three sternites the dose resulting in 50% morphological inhibition of metamorphosis (ID_{50}Morph., Slama, 1971) is 5 μg/g live weight. The ID_{50}Morph. for topically applied JH is about 8 μg/g for Tenebrio, and 25 μg/g for *Pyrrhocoris* (Slama, 1971; Williams, 1970). It therefore appears that the *Drosophlia* abdomen is about as sensitive to JH as other insects.

In many insect orders the male genitalia rotate to accommodate the female during copulation. In Diptera the rotation occurs during development and is permanent. This rotation involves the abdominal tissue surrounding the genitalia rather than the genitalia *per se* (Gleichauf,, 1936; Scudder, 1971). One of the effects noted after JH application to *Drosophila* is failure of the male genitalia to complete rotation (Bryant and Sang, 1968). Failure to complete rotation occurs also after treatment of the mosquito *Aedes aegypti* (Spielman and Williams, 1966) and *Sarcophaga* (Bhaskavan, 1972) with JH analogues. The JH sensitive stage for the rotation in *Drosophila* is from shortly before pupariation until about 15 hours (Fig. 5B). This sensitive phase is well prior to the actual time of rotation for *Drosophila*, which does not occur until the late stages of metamorphosis (Gleichauf, 1936).

These results confirm that the abdomen is quite sensitive to JH while the head and thorax are not and the differentiation of the genitalia is less sensitive than the abdomen (Dearden, 1964; Bryant and Sang, 1969; Ashburner, 1970b; Madhavan, 1973). This difference is probably not due to the method of application since treatment on the anterior or posterior gives similar results and removal of the operculum to allow application of hormone directly to the pupal head does not cause the head and thorax to be affected. In addition, feeding the hormone after the method of Bryant and Sang (1969) elicits the same syndrome of abnormalilities which are described here for topical application.

If the difference in response of different tissues is not an artefact it must have some basis in the developmental physiology of various body parts. The development of the epidermis of the imaginal abdomen differs from the development of the head, thorax, and genitalia in two significant respects. First, the adult abdomen of higher flies arises from clusters of histoblasts, which undergo little or no cell division from embryogenesis until the time of pupariation (Robertson, 1936; Garcia-Bellido and Merriam, 1971a; Guerra, Postlethwait and Schneiderman, 1973). The other integumentary structures arise during embryogenesis as sacs of cells, called imaginal discs. The imaginal disc cells proliferate rapidly during larval life, but slow down after pupariation (Becker, 1957; Bryant and Schneiderman, 1969; Bryant, 1970; Garcia-Bellido and Merriam, 1971b; Postlethwait and Schneiderman, 1971; Ulrich, 1971). Thus, when cell division rate is high in the abdomen, cell division rate is low in the other imaginal anlagen. Our results (Fig. 5) show that the time of JH sensitivity for the abdomen is precisely the time of rapid proliferation of abdominal histoblast cells.

A correspondence of JH sensitivity and high proliferation rate appears to be a general phenomenon in the insect integument. For example, in *Rhodnius*, sensitivity to exogenously supplied JH analogues is greatest at 8 days after feeding, which is the time mitosis is beginning in the epidermis (Wigglesworth, 1963). In some cases an increased proliferation rate can be induced experimentally. Piepho and Heims (1952) demonstrated that regenerating cells of the waxmoth *Galleria mellonella* are much more sensitive to JH than undividing cells. This fact has been exploited in the very sensitive "wax test" for JH activity (Schneiderman and Gilbert, 1958).

The second main difference between abdominal and anterior tissues is that the pupal cuticle of the abdomen is secreted at least largely by the larval abdominal cells, while the pupal cuticle of the anterior is secreted by the imaginal disc cells. So it is not clear whether the aberrant (pupal-like) adult cuticle in *Drosophila* is secreted by larval or imaginal cells. It is clear for *Sarcophaga* that the aberrant adult cuticle is secreted by imaginal cells (Bhaskaran, 1971). Further histological studies are in progress to answer this question for *Drosophila*.

The correspondence between cell division and JH activity has been thought to be fundamental to the action of the hormone. Williams and Kafatos (1971) have proposed that cell division in the presence of JH delays the activation of new gene sets, whereas cell division in the absence of JH permits new programs of genetic information to be utilized. Perhaps further hormonal research on *Drosophila*, in which genetics can be effectively used to investigate developmental problems, will lead to a better understanding of such problems.

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Summary

Topical application of Cecropia juvenile hormone (JH) blocked metamorphosis of the abdominal integument of pupating *Drosophila* larvae. JH treated adult flies retained more or less extensive areas of cuticle which could not be distinguished from pupal cuticle in whole mounts. Implantation into mature larvae of fragments of first instar larvae containing brains and ring glands provoked localized retention of patches of incompletely metamorphosed cuticle. An assay procedure for compounds with juvenile hormone activity was developed by defining the critical developmental stage and dose-response relationships. Twenty-two substances suspected of having juvenile hormone activity were assayed for their effect on *Drosophila*. These findings support the choice of *Drosophila* for resolving the genetic basis of juvenile hormone action.

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